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Transcytotic pathway for blood-borne protein through the blood–brain barrier

(endothelium/wheat germ agglutinin/endocytosis/exocytosis/Golgi complex)

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ABSTRACT **The transcytosis of blood-borne protein through the blood–brain barrier, a consequence of recruitment of the Golgi complex within nonfenestrated cerebral endothelia, was identified in mice and rats injected intravenously with the lectin wheat germ agglutinin (WGA) conjugated to the enzymatic tracer horseradish peroxidase (HRP). WGA enters cells by adsorptive endocytosis after binding to specific cell surface oligosaccharides. Blood-borne WGA-HRP labeled the entire cerebrovascular tree from the luminal side 5 min after injection; pericytes, located on the abluminal surface of cerebral endothelia, sequestered the lectin conjugate 6 hr later. Endothelial organelles harboring WGA-HRP 3 hr after injection included the luminal plasmalemma, endocytic vesicles, endosomes (prelysosomes), secondary lysosomes, and the Golgi complex. The peroxidase reaction product labeled the abluminal surface of cerebral endothelia and occupied the perivascular clefts by 6 hr. Within 12 hr, organelles labeled with WGA-HRP in pericytes were identical to those observed in endothelia. Blood-borne native HRP, entering cells by bulk-phase endocytosis, was neither directed to the Golgi complex nor transferred across nonfenestrated cerebral endothelia. The results suggest that blood-borne molecules taken into the cerebral endothelium by adsorptive endocytosis and conveyed to the Golgi complex can, either by themselves or as vehicles for other molecules excluded from the brain, undergo transcytosis through the blood–brain barrier without compromising the integrity of the barrier.**

Evidence for the transcytosis (endocytosis to intracellular transport to exocytosis) of blood-borne protein through nonfenestrated cerebral endothelia (blood–brain barrier) is largely morphological and is based on application of the probe molecule native horseradish peroxidase (HRP); native HRP enters cells indiscriminately by fluid or bulk-phase endocytosis. Studies employing HRP suggest that transendothelial vesicular transport of the protein occurs normally from blood to brain through segments of specific arterioles (1) and from brain to blood through capillaries (2, 3). Other investigations propose that transcytosis of blood-borne peroxidase through cerebral capillaries is induced experimentally (4–7). The fusion of intraendothelial vesicles to form patent transendothelial channels is documented for fenestrated endothelia in circumventricular organs (8); however, existence of similar channels within the blood–brain barrier endothelia is equivocal (9).

Our investigations of the mammalian blood–brain barrier have emphasized that blood-borne native HRP with internalized endothelial surface membrane is directed to endosomes (a prelysosomal compartment) and to secondary lysosomes for eventual degradation without undergoing transendothelial transport (9–13). Protein tracers exposed to the abluminal surface of the cerebral endothelium by way of the blood through the meninges in the mouse, by ruptured interendothelial tight junctions, or by ventriculo-cisternal perfusion of the proteins are not endocytosed demonstrably by the endothelium but fill stationary pits and invaginations in the abluminal surface (9, 11–13). We speculated that the abluminal pits are misinterpreted by others as vesicles participating in transendothelial transport bidirectionally through the cerebral endothelium (11, 13). A similar interpretation is available for endothelial cells in peripheral tissues (14). Failure of the cerebral endothelium to endocytose protein tracers bathing its abluminal surface suggests that the cerebral endothelium is polarized with regard to retrieval of its cell surface membrane and argues for a *brain–blood barrier*. Nevertheless, the possibility cannot be dismissed that a population of abluminal surface pits represents exocytic vesicles that have fused with the plasmalemma.

This report extends our evaluation of membrane events associated with the mammalian cerebral endothelium and offers the first conclusive morphological evidence for the transcytosis of a blood-borne protein through the blood–brain barrier. The protein used was the plant lectin wheat germ agglutinin (WGA) that was conjugated to HRP as the tracer and administered intravenously to mice and rats. The transcytosis of blood-borne WGA-HRP through nonfenestrated cerebral endothelia is believed to be attributed to inclusion of the Golgi complex in the transcytotic pathway. This and other potential intraendothelial routes that blood-borne fluid-phase macromolecules, lectins, and ligands (e.g., transferrin, insulin, peptides, etc.) may utilize in traversing the blood–brain barrier are discussed and summarized diagrammatically.

MATERIALS AND METHODS

Twenty-one Swiss–Webster mice and seven Sprague–Dawley rats of both sexes were used in this study. Twenty mice and six rats were anesthetized with Nembutal and injected into the jugular vein with 0.2–0.8% WGA-HRP (70 kDa; Sigma) in saline; mice received 0.25 ml, and rats received 0.5 ml of the WGA-HRP/saline mixture. The lectin was prepared by the method of O'Sullivan *et al.* (15) and was affinity purified after conjugation. We reported (16) that WGA-HRP is fatal to mice when delivered rapidly as an intravenous bolus. Death was considered the result of the protein initiating the agglutination of erythrocytes. When WGA-HRP is administered slowly into the jugular vein, the protein is accepted without difficulty by mice and rats. Survival times after injection of the animals ranged from 5 min to 24 hr. Blood vessels supplying cerebral gray and white matter, the neurohypophysis (median eminence and posterior lobe of the pituitary gland), choroid plexus, and anterior pituitary

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Abbreviations: HRP, horseradish peroxidase; WGA, wheat germ agglutinin.

gland were analyzed by light and electron microscopy after tissue incubation in a medium containing diaminobenzidine or tetramethylbenzidine as the chromogen. Brains and pituitary glands from one noninjected mouse and one noninjected rat were inspected for endogenous peroxidase activity; such activity was evident only in ependymal cells and glia of the hypothalamic arcuate nucleus. Detailed descriptions of the perfusion/fixation of the animals and preparations of the brains for peroxidase cytochemistry at the light microscopic and ultrastructural levels are provided elsewhere (12, 17, 18).

RESULTS

None of the animals injected intravenously with WGA-HRP died from the injection, and none demonstrated observable adverse physical or physiological responses to the protein upon regaining consciousness from the anesthesia.

Tetramethylbenzidine and diaminobenzidine were equally reliable at the light microscopic level for revealing the location of blood-borne WGA-HRP in brains of injected animals (18). Reaction product outlined cerebral blood vessels throughout the gray and white matter (Fig. 1*A*) and all circumventricular organs within 5 min of the injection. At 1 hr, the parenchyma of the neurohypophysis and that of the anterior pituitary lobe were inundated with reaction product, suggesting that the blood-borne lectin conjugate escaped fenestrated vessels supplying these sites (19). Conversely, fenestrated vessels of the choroid plexus, subfornical organ, and area postrema appeared impermeable to blood-borne WGA-HRP through 24 hr. Reaction product labeled the dura mater but never appeared on the pial surface or within the parenchyma adjacent to the circumventricular organs. Extravasations of blood-borne WGA-HRP within the brain parenchyma were absent; thus, the blood–brain barrier was not compromised by injection of the protein or by the protein itself. Pericytes related to superficial and deep cerebral vessels were labeled with WGA-HRP between 6 and 24 hr after injection (Fig. 2*A*).

Nonfenestrated and fenestrated endothelia exposed to blood-borne WGA-HRP for 3 hr or less were compared ultrastructurally to identical endothelia exposed for 6 hr or more. The comparison was derived from an analysis of 128 endothelial cells sampled from widespread areas in the brain and from the choroid plexus, neurohypophysis, and anterior pituitary lobe. Distinct differences were observed in the intracellular and extracellular localizations of peroxidase reaction product. From 5 min to 3 hr, reaction product coated the luminal surface of nonfenestrated, cerebral endothelia and was identified within the following endothelial organelles: endocytic vesicles measuring 40–70 nm in diameter, which often were clustered around 0.2- to 0.8-μm-wide spherical vacuoles rimmed internally with peroxidase reaction product (endosomes), tubular profiles, dense and multivesicular bodies, and one or two peripheral saccules of the Golgi complex (Fig. 1 *B*, *C*, and *D*). With the exception of the Golgi complex, the same types of endothelial organelles were labeled with peroxidase reaction product in animals receiving native HRP intravenously (9–13). Populations of the tubular profiles, multivesicular bodies, and dense bodies are representative of secondary lysosomes based on application of acid hydrolase cytochemistry (9, 10). Reaction product for blood-borne WGA-HRP was not observed in the perivascular clefts or on the abluminal surface of nonfenestrated, cerebral endothelia for up to 3 hr after injection (Fig. 1 *B*, *C*, and *D*) but was evident between 6 and 24 hr (Figs. 1*E* and 2*B*). Reaction product at 6 hr and longer also was seen on the luminal surface membrane and within endothelial vesicles, endosomes, tubules, and multivesicular and dense bodies (Figs. 1*E* and 2*B*). Pericytes adjacent to the abluminal surface of endothelia close to or far removed from the pial surface and circumventricular organs contained the same complement of organelles labeled with WGA-HRP 12 hr after injection (Fig. 2 *B* and *C*) that was identified within endothelial cells. Extracellular reaction product in the neuropil was not conspicuous beyond the endothelia and pericytes (Figs. 1*E* and 2*B*).

Peroxidase reaction product for blood-borne WGA-HRP was identified in the perivascular and extracellular clefts of the neurohypophysis and anterior pituitary gland prior to 1 hr after injection. The luminal surface of fenestrated endothelia supplying the choroid plexus was coated with reaction product of WGA-HRP 3 hr after injection of the lectin conjugate; 6 hr and longer after injection, reaction product appeared on the abluminal surface of these endothelia and within endosomes, multivesicular bodies, and dense bodies in endothelia and demonstrably less so in epithelia of the choroid plexus. A Golgi complex was not recognized in endothelia of the choroid plexus, and peroxidase reaction product was not evident in Golgi saccules of the choroid plexus epithelia. Endocytic activity of fenestrated capillaries in the neurohypophysis and anterior pituitary gland was not apparent. Exposure of anterior pituitary and neurosecretory cells to blood-borne WGA-HRP was confirmed by identification of peroxidase reaction product within the trans-most Golgi saccule and secretory granules derived from this Golgi saccule (19) in these cell types.

All fenestrated and nonfenestrated endothelia, pericytes, and other cells exposed to blood-borne WGA-HRP and examined ultrastructurally appeared undamaged and exhibited intact plasma membranes. Peroxidase reaction product was never seen within the cytoplasmic matrix of the cells.

DISCUSSION

This study provides conclusive morphological evidence for the transendothelial transfer of a blood-borne protein through the blood–brain barrier. The protein used was WGA, a plant lectin that binds to cell surface sialic acid and *N*-acetylglucosamine and enters cells by the process of adsorptive endocytosis (20). Blood-borne WGA-HRP is exposed to and endocytosed by nonfenestrated endothelia throughout the central nervous system. In time, the internalized lectin conjugate is conveyed to the Golgi complex, most likely to the innermost Golgi saccule, wherein the protein is packaged for export within and for eventual exocytosis from the cerebral endothelium. Intact WGA-HRP is directed, in part, to the abluminal side of cerebral endothelia and released into the perivascular clefts as exemplified by the adsorptive endocytosis of the molecule by pericytes. Our results suggest that blood-borne molecules entering cerebral endothelia by adsorptive endocytosis and channeled to the Golgi complex can undergo transcytosis through the blood–brain barrier without compromising the integrity of the barrier. These molecules may potentially serve as vehicles for the delivery of blood-borne chemotherapeutics (e.g., *L*-dopa, peptides, enzymes, etc.) through the blood–brain barrier into the brain. The transcytosis of protein through the cerebral endothelium may be *vectorial*, since the luminal surface but not the abluminal surface exhibits demonstrable endocytic activity (11, 13).

Adsorptive endocytosis associated with inclusion of the Golgi complex in the transendothelial pathway is responsible for the transcytosis of blood-borne WGA-HRP. Blood-borne native HRP taken into nonfenestrated cerebral endothelia by bulk-phase endocytosis is not directed to the Golgi complex and is not transferred across the blood–brain barrier (9–13). Other data obtained from our laboratory (16) offer similar interpretations to explain the transsynaptic transfer of native WGA (21) and of WGA-HRP (22, 23) and the absence of native HRP transfer between neurons. Additional lectin conjugates (e.g., ricin, cholera toxin, and phytohemaggluti-

nin) and cationized ferritin, all of which enter cells by adsorptive endocytosis, also are directed to the Golgi complex (24–26). Sequestration of these molecules within the Golgi complex is restricted primarily to the Golgi saccule that is cytochemically acid phosphatase positive and located at the inner or trans face of the Golgi stack (16, 27, 28). The same Golgi saccule is involved in the packaging of membrane and endogenous molecules destined for export to multiple sites within the cell and in the packaging of secretory material to be released from the cell (29).

Our data do not permit a precise delineation of the Golgi transcytotic pathway through the cerebral endothelium. Peroxidase labeling of the inner Golgi saccule occurs after endosomal labeling but precedes the appearance of peroxidase reaction product on the abluminal side of the endothelium. We cannot interpret with certainty whether WGA-

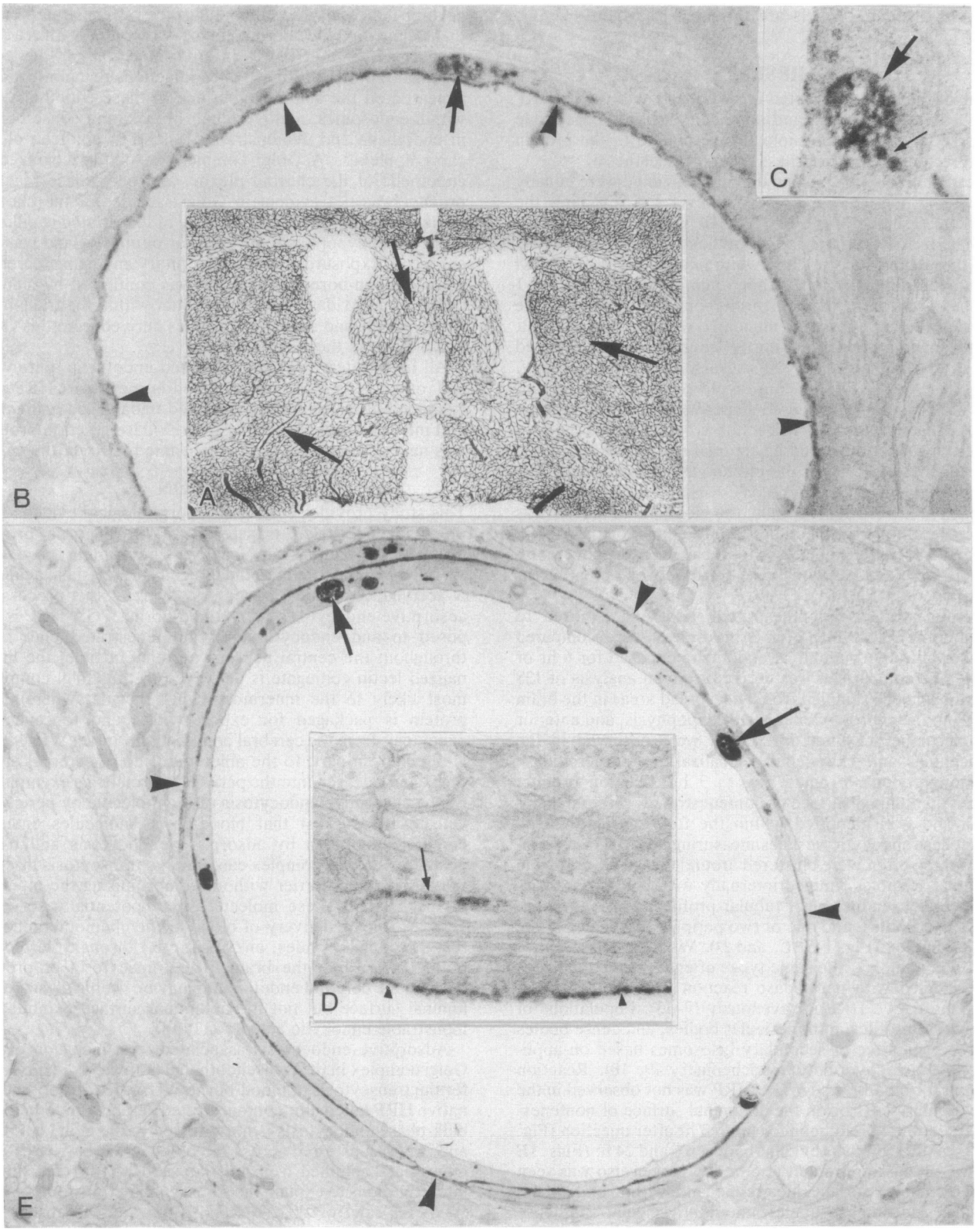

FIG. 1. *(Legend appears at the bottom of the opposite page.)*

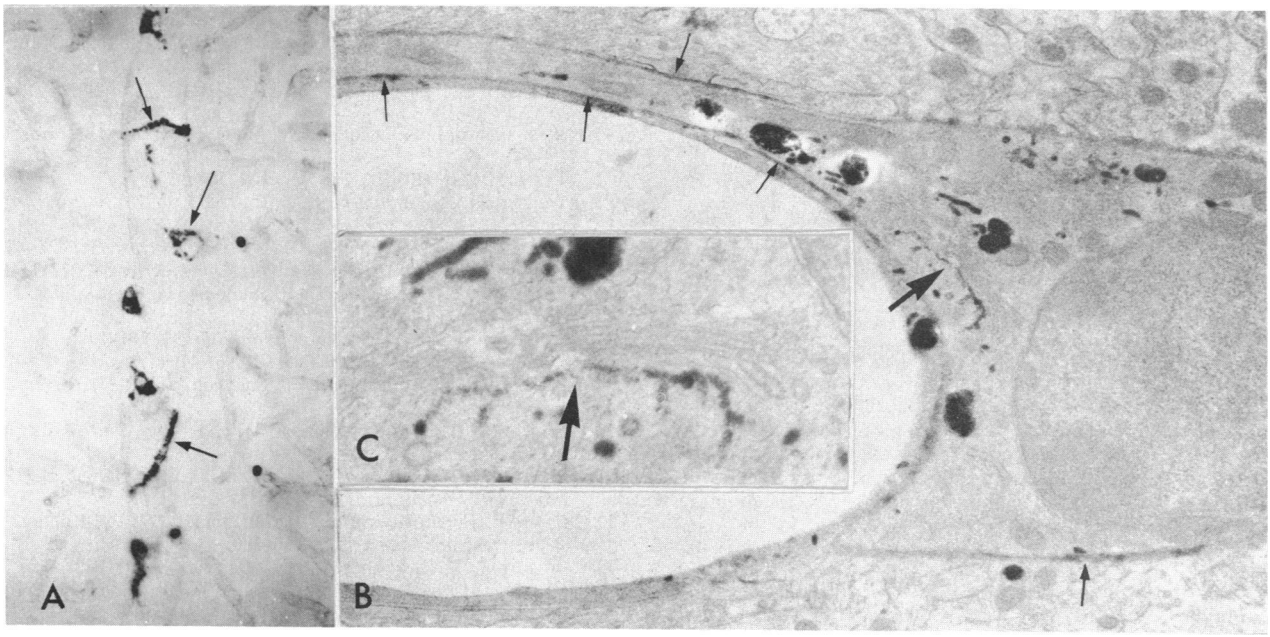

FIG. 2. Blood-borne WGA-HRP in the mouse labeled cerebral pericytes (*A*, arrows) between 6 and 24 hr after injection. Reaction product was identified ultrastructurally within the extracellular clefts surrounding endothelial cells and pericytes (*B*, small arrows). Pericyte endosomes and dense bodies as well as the inner saccule of the Golgi complex (*B* and *C*, large arrows) also exhibited peroxidase reaction product. (*A*, ×775; *B*, ×14,250; *C*, ×38,000.)

HRP with associated membrane reaches the Golgi complex directly from the cell surface or indirectly by way of endosomes. Similarly, we do not know if WGA-HRP is delivered to the abluminal plasmalemma of cerebral endothelia in transfer vesicles originating from endosomes, from the Golgi complex, or from both. The time frame for appearance of peroxidase reaction product within the perivascular clefts (i.e., >3 hr after injection) suggests that the transcytotic pathway for blood-borne WGA-HRP utilizing endocytic and transfer vesicles within nonfenestrated, cerebral endothelia is the following: luminal plasmalemma to endosomes to transmost Golgi saccule to abluminal plasmalemma (Fig. 3). Some transport vesicles derived from the inner Golgi saccule may provide new membrane for the luminal and abluminal walls of the endothelial cell; other Golgi vesicles may represent primary lysosomes engaged in ferrying acid hydrolases to endosomes and secondary lysosomes (Fig. 3). A similar transcytotic pathway may convey blood-borne WGA-HRP through choroid plexus fenestrated endothelia that are not readily permeable to this protein.

Why the Golgi complex sequesters WGA-HRP is unclear. Because endosomes occupy a nodal point in the endocytic pathway within cells in general (see below), we suggest that the ability of endosomes to dissociate lectin from internalized surface membrane is compromised when massive retrieval of the plasmalemma labeled with lectin occurs. An alternative endocytic pathway may be established through which lectin-labeled membrane is transferred to the inner Golgi saccule from endosomes and/or directly from the cell surface. The inner Golgi saccule may participate in the remodeling of lectin-labeled membrane. Sialic acid residues, to which WGA binds on the cell surface, are added to membrane glycoprotein in the trans-most Golgi saccule (32, 33). The dissociation of WGA from membrane cycled through the inner Golgi saccule would permit the packaging of WGA-HRP within Golgi derived vesicles for export and exocytosis. Absence of blood-borne WGA-HRP in the Golgi complex of choroid plexus epithelia may be attributed to the ability of the endosome compartment in this cell to dissociate lectin from internalized lectin-labeled plasmalemma (34).

The physiological/pharmacological significance of the Golgi transcytotic pathway for blood-borne molecules traversing the blood–brain barrier is questionable given the lengthy transport time required. Nevertheless, the Golgi complex in a host of cell types has been proposed as the site where ligands and membrane components (receptors) are modified or repaired during recycling through Golgi saccules; thus, the Golgi complex may exert significant influence on membrane equilibria (24, 35). Available data suggest that the transferrin receptor in two different cell lines recycles through Golgi saccules (36, 37) and that receptor-mediated transcytosis of transferrin can occur through cerebral endothelia (38). The exact intraendothelial pathway for a receptor-mediated transcytosis of transferrin and insulin (39, 40) through the blood–brain barrier remains to be clarified.

FIG. 1. (*Opposite page*) (*A*) WGA-HRP delivered intravenously to the mouse and permitted to circulate for 5 to 30 min labeled macro and micro blood vessels (arrows) throughout the brain. Unlike blood-borne native HRP in the mouse (see ref. 12), WGA-HRP failed to reach the pial surface extracellularly from leaky vessels supplying the dura mater. (×40.) Three hours after the intravenous injection of WGA-HRP in the mouse, peroxidase reaction product coated the luminal surface of the endothelium (*B*, large arrow) and was localized in endothelial vesicles (*C*, small arrow) clustered around endosomes (*C*, large arrow) and in multivesicular bodies and dense bodies (*B*, large arrow). The abluminal face of the endothelium and perivascular clefts did not exhibit reaction product at this time. (*B*, ×30,000; *C*, ×45,000.) (*D*) A saccule of the Golgi complex (arrow) in a cerebral endothelial cell was labeled with peroxidase reaction product 3 hr after intravenous injection of WGA-HRP in the mouse; reaction product was visible on the luminal surface (arrowheads) but absent on the abluminal surface of this cell. (×58,500.) (*E*) Six to 12 hr after intravenous injection of WGA-HRP in mouse and rat, peroxidase reaction product was evident on the abluminal surface and/or within the perivascular clefts (large arrowheads) of cerebral endothelia. The section shown is from a rat. The luminal surface membrane and endothelial organelles (large arrows) identical to those seen in *B*, *C*, and *D* also were labeled with reaction product. (×10,000.)

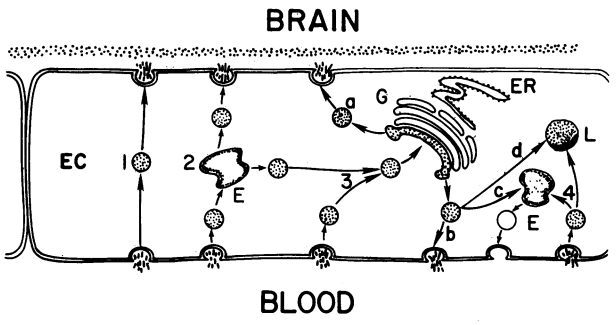

FIG. 3. Hypothetical transcytotic pathways for blood-borne macromolecules through the nonfenestrated, cerebral endothelial cell (EC) include the following. Pathways: 1, endocytic vesicles may ferry molecules directly from the luminal to the abluminal side of the endothelium; 2, endocytic vesicles derived from the luminal surface membrane are directed to endosomes (E) that give rise to exocytic vesicles directed to the abluminal membrane; 3, vesicles from the luminal plasmalemma or endosomes are channeled to the inner Golgi saccule (G). Transport vesicles from the inner Golgi saccule may engage in exocytosis at the abluminal (pathway a) and luminal (pathway b) cell surfaces, or they may represent primary lysosomes charged with delivering acid hydrolases to endosomes (pathway c) and secondary lysosomes (L; pathway d). Internalized cell surface membrane that may or may not contain receptor sites is usually directed to secondary lysosomes or to endosomes; the latter may recycle membrane harboring receptor sites to the cell surface (pathway 4; see refs. 30 and 31). These four intracellular pathways may apply to blood-borne ligands internalized within cerebral endothelia by receptor-mediated endocytosis. Because endocytosis appears not to occur at the abluminal side (11, 13), the blood–brain barrier may be polarized with regard to the transcytosis of protein. ER, endoplasmic reticulum.

Potential transendothelial pathways anticipated to offer shorter transport time and greater efficiency than the Golgi transcytotic route involve direct vesicular transport from the luminal to abluminal side and vesicular transport by way of endosomes (Fig. 3). Direct transendothelial vesicular transport through cerebral and noncerebral endothelia is speculative at the present time. This pathway is reported for the receptor-mediated transcytosis of gold-labeled albumin in peripheral endothelial cells (41). Analyses of cerebral and noncerebral endothelia in which the luminal and abluminal plasma membranes appear attenuated suggest that a coalescence of vesicles may form transendothelial channels through which blood-borne molecules can pass unobstructed (8, 41). Endosomes serving as an intermediary in the transcellular vesicular transport of ligands (Fig. 3, pathway 2) is documented for the intestinal epithelium (42) and may be represented similarly in the transcytosis of WGA-HRP through choroid plexus epithelia from the cerebrospinal fluid; in the latter cell type, transcytosis of WGA-HRP occurs within 10 min (34).

Molecules internalized in endocytic vesicles in most cell types are directed to endosomes, a prelysosomal compartment representing a clearing center and first intracellular stop for fluid phase macromolecules, lectins, and ligands entering cells by bulk phase, adsorptive, and receptor-mediated endocytic processes, respectively (30, 43). In lieu of endosomes, secondary lysosomes (e.g., multivesicular and dense bodies) also can receive internalized macromolecules and cell surface membrane (Fig. 3). Transport vesicles arising from endosomes may complete the transcytotic pathway through the cell or, as we have reported for neurons (16, 44), anterior pituitary cells (19), and now cerebral endothelia, may be directed to the trans-most Golgi saccule where additional transporting/exocytic vesicles take origin.

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1. Westergaard, E. & Brightman, M. W. (1973) *J. Comp. Neurol.* **152,** 17–44.
2. Van Deurs, B. (1977) *Brain Res.* **124,** 1–8.
3. Wagner, H. J., Pilgrim, C. L. & Brandl, J. (1974) *Acta Neuropathol.* **27,** 299–315.
4. Beggs, I. L. & Waggener, J. D. (1976) *Lab. Invest.* **34,** 428–439.
5. Petito, C. K. (1979) *J. Neuropathol. Exp. Neurol.* **38,** 222–234.
6. Povlishock, J. T., Becker, D. P., Sullivan, H. G. & Miller, J. D. (1978) *Brain Res.* **153,** 222–239.
7. Westergaard, E. (1977) *Acta Neuropathol.* **39,** 181–187.
8. Coomber, B. L. & Stewart, P. A. (1986) *Anat. Rec.* **215,** 256–261.
9. Balin, B. J., Broadwell, R. D. & Salcman, M. (1987) *J. Neurocytol.*, in press.
10. Broadwell, R. D. & Salcman, M. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 7820–7824.
11. Broadwell, R. D., Balin, B. J., Salcman, M. & Kaplan, R. S. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 7352–7356.
12. Balin, B. J., Broadwell, R. D., Salcman, M. & El-Kalliny, M. (1986) *J. Comp. Neurol.* **251,** 260–280.
13. Broadwell, R. D., Balin, B. J. & Salcman, M. (1987) *Wiss. Z. Karl-Marx-Univ. Leipzig Meth. Naturwiss. Reihe* **36,** 170–174.
14. Bundgaard, M. (1983) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **42,** 4225–4230.
15. O'Sullivan, M. J., Gnemmi, E., Morris, D., Chieregatti, G., Simmons, M., Simmonds, A. D., Bridges, J. W. & Marks, V. (1978) *FEBS Lett.* **95,** 311–313.
16. Broadwell, R. D. & Balin, B. J. (1985) *J. Comp. Neurol.* **242,** 632–650.
17. Broadwell, R. D. & Brightman, M. W. (1983) *Methods Enzymol.* **103,** 187–218.
18. Broadwell, R. D., Charlton, H. M., Balin, B. J. & Salcman, M. (1987) *J. Comp. Neurol.* **60,** 47–62.
19. Balin, B. J. & Broadwell, R. D. (1987) *J. Histochem. Cytochem.* **35,** 489–498.
20. Gonatas, N. K. & Avrameas, S. (1973) *J. Cell Biol.* **59,** 436–443.
21. Ruda, M. & Coulter, J. D. (1982) *Brain Res.* **249,** 237–246.
22. Schwab, M. E., Suda, K. & Thonen, H. (1979) *J. Cell Biol.* **82,** 798–810.
23. Trojanowski, J. Q. & Schmidt, M. L. (1984) *Brain Res.* **311,** 366–369.
24. Gonatas, N. K. (1982) *J. Neuropathol. Exp. Neurol.* **41,** 6–17.
25. Gonatas, N. K. & Gonatas, J. O. (1983) *Immunoenzyme Techniques*, ed. Avrameas, S. (Elsevier, Amsterdam), pp. 111–120.
26. Farquhar, M. G. (1978) *J. Cell Biol.* **77,** 435–442.
27. Gonatas, N. K., Kim, S. U., Steiber, A. & Avrameas, S. (1977) *J. Cell Biol.* **73,** 1–13.
28. Broadwell, R. D. & Oliver, C. (1983) *J. Histochem. Cytochem.* **31,** 325–335.
29. Griffiths, G. & Simmons, K. (1986) *Science* **234,** 438–443.
30. Helenius, A., Mellman, I., Wall, D. & Hubbard, A. (1983) *Trends Biochem. Sci.* **8,** 245–249.
31. Dautry-Varsat, A. & Lodish, H. F. (1984) *Sci. Am.* **250,** 52–58.
32. Bennett, G. & O'Shaughnessy, D. (1981) *J. Cell Biol.* **88,** 1–15.
33. Bennett, G., Kan, F. W. K. & O'Shaughnessy, D. (1981) *J. Cell Biol.* **88,** 16–28.
34. Balin, B. J., Broadwell, R. D. & Salcman, M. (1987) *Soc. Neurosci. Abstr.* **131,** 1250.
35. Farquhar, M. G. (1983) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **42,** 2407–2413.
36. Snider, M. D. & Rogers, O. C. (1985) *J. Cell Biol.* **100,** 826–834.
37. Woods, J. W., Doriaux, M. & Farquhar, M. G. (1986) *J. Cell Biol.* **103,** 277–286.
38. Fishman, J. B., Handrahan, J. V., Connor, J., Dickey, B. F. & Fine, R. E. (1985) *J. Cell Biol.* **423a,** 1592 (abstr.).
39. Pardridge, W. M., Eisenberg, J. & Yang, J. (1985) *J. Neurochem.* **44,** 1771–1778.
40. Duffy, K. R. & Pardridge, W. M. (1987) *Brain Res.* **420,** 32–38.
41. Simionescu, M., Ghitescu, L., Fixman, A. & Simionescu, N. (1987) *News Physiol. Sci.* **2,** 97–100.
42. Rodewald, R. & Kraenbuhl, J.-P. (1984) *J. Cell Biol.* **99,** 159s–164s.
43. Steinman, R. M., Mellman, I. S., Muller, W. A. & Cohn, Z. A. (1983) *J. Cell Biol.* **96,** 1–27.
44. Broadwell, R. D., Cataldo, A. M. & Balin, B. J. (1984) *J. Comp. Neurol.* **288,** 155–167.